INTERACTION-ACTIVATED β-LACTAMASES

ABSTRACT OF THE DISCLOSURE

5

Fragment pairs of a Class A β-lactamase (TEM-1 of *E. coli*) are disclosed that depend for their functional reassembly into the parent protein on the interaction of heterologous polypeptides or other molecules which have been genetically or chemically conjugated to the break-point termini of the fragment pairs. In addition, methods are provided for identifying fragment pairs that will optimally reassemble into a functional parent protein. Fragment pairs that comprise molecular interaction-dependent enzymes find use in (1) homogeneous assays and biosensors for any analyte having two or more independent binding sites, (2) tissue-localized activation of therapeutic and imaging reagents *in vivo* for early detection and treatment of cancer, chronic inflammation, atherosclerosis, amyloidosis, infection, transplant rejection, and other pathologies, (3) cell-based sensors for activation or inhibition of metabolic or signal transduction pathways for high-efficiency, high-throughput screening for agonists/antagonists of the target pathway, (4) high-throughput mapping of pair-wise protein-protein interactions within and between the proteomes of cells, tissues, and pathogenic organisms, (5) rapid selection of antibody fragments or other binding proteins which bind specifically to polypeptides of interest, (6) rapid antigen identification for anti-cell and anti-tissue antibodies, (7) rapid epitope identification for antibodies, (10) cell-based screens for high-throughput selection of inhibitors of any protein-protein interaction.

10

15

20

25